Measuring the Elastic Properties of Thin Polymer Films with the Atomic Force Microscope

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The elastic properties of thin gelatin films were investigated with the atomic force microscope (AFM). The degree of swelling and thus the softness of the gelatin can be tuned by immersing it in mixtures of propanol and water. Therefore, we have chosen gelatin films as a model system to characterize the measurement of elasticity of thin and soft samples. The major aim of this study was to investigate the influence of the film thickness on the apparent elastic (Young's) modulus. Thus, we prepared wedge-shaped samples with a well-defined thickness of up to 1 μm. The Young's modulus of our samples was between 1 MPa and 20 kPa depending on the degree of swelling. The elasticity was calculated by analyzing the recorded force curves with the help of the Hertz model. We show that the calculated Young's modulus is dependent on the local film thickness and the applied loading force of the AFM tip. Thus, the influence of the hard substrate on the calculated softness of the film can be characterized as a function of indentation. It was possible to determine the elastic properties of gelatin films with a thickness down to 50 nm and a Young's modulus of ~20 kPa.

Introduction

The atomic force microscope (AFM)[1,2] is a rather new and powerful method for studying biological samples. With the ability of imaging proteins, DNA, or even whole cells in their physiological environment,[3,4] the AFM is an important tool for the investigation of biological processes on the nanometer scale. The AFM can not only be used for imaging the topography of surfaces but also for measuring forces on the molecular level. The AFM has been used for measuring binding forces between single molecules[5] and for observing conformational changes when stretching single molecules.[6] Because it is also possible to apply well-defined small forces on a sample, the AFM was soon used as a nanoindenter measuring elastic properties,[7] this method has also been applied to biological samples.[8−10] For investigating the elasticity, force curves are taken: the sample is compressed by the indenting AFM tip and the elastic response of the sample under this loading force is analyzed. The so-called force-mapping mode is a very sensitive tool for measuring interaction forces such as adhesion[11] or electrostatics[12] with a lateral resolution of only a few nanometers. When applying this technique to living cells,[13,14] the combination of an extremely soft and very thin sample of only a few hundreds of nanometers in the peripheral parts creates a serious problem; that is, the AFM tip can completely compress the sample. Thus, the tip will "feel" the underlying stiff substrate. Consequently, the calculated or apparent Young's modulus is too high and the sample appears too stiff. There are theoretical models describing the elastic behavior of thin films when indented by a sphere[15] or a cylinder.[16] However, to our knowledge there is no model for the most appropriate geometry for an AFM tip; that is, a cone or even a cone with a half sphere added at the end. Therefore, we chose to elucidate this problem by developing an experimental system that can be controlled very well. As a model system we picked thin gelatin films for several reasons. This polymer is well investigated because of its use in the food and photographic industries[17,18] and it has previously been investigated with the AFM.[19] Moreover, sample preparation is rather simple. We prepared wedges with a thickness of up to 1 μm to analyze the dependence of thickness on the measured Young's modulus. Additionally, the softness of the gel could be tuned by immersing it in different mixtures of propanol and water[10] so that it was possible to adjust thickness and softness of our sample in a way that we could model the experimental conditions we also find in living cells.

Materials and Methods

Sample Preparation. Gelatin pork skin with a gel strength of 250 Bloom was purchased from Fluka (Deisenhofen, FRG). A 100-mg amount of gelatin was dissolved in 20 mL of pure water

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(Milli-Q quality, MilliPore, Molsheim, FRG) at a temperature of 50 °C to obtain a concentration of 5 mg/mL, which corresponds to the minimum concentration for yielding a cross-linked gelatin network.[20] We added 10 droplets of 2 mg/mL Coomasie blue (Brilliant Blue R, Sigma, Deisenhofen, FRG) for improving the visibility of the edge of the gelatin in the optical microscope. After 15 min of stirring, two droplets of the solution were put on a precleaned microscope slide (Sigma) and were air-dried overnight. Typically, samples were used the next day. However, occasionally we used them after weeks of storage without observing any differences. For the experiments, the samples were first immersed in pure 1-propanol (Sigma, HPLC grade). Then we added water gradually up to a final concentration of ~60% to induce swelling. All AFM measurements were done in a fluid environment.

Instrumentation. The AFM studies were performed with a commercial instrument (Bioscope, Digital Instruments, Santa Barbara, CA) mounted on an inverted optical microscope (Zeiss Axiomat, Zeiss, Oberkochen, FRG). This combination enabled us to position the AFM tip on the area of interest of our sample, in our case right at the edge of the gelatin droplet. We used silicon nitride cantilevers (Sharpened Microlevers, Park Scientific, Santa Clara, CA) with a force constant of 8 mN/m. Cantilevers were calibrated by measuring the thermally induced motion of the unloaded cantilever.[21]

Data Analysis of the Force Curves. The slope of a force curve already describes the elastic properties of a sample in a qualitative way. On an infinitely stiff sample, the deflection d of the cantilever is identical to the movement of the piezo in z direction: $d = z$. In the case of a soft sample, the cantilever tip will indent the sample. This indentation δ leads to a smaller deflection $d = z - \delta$, resulting in a flatter force curve with a smaller slope.

Because Hooke's law connects the deflection of the cantilever and the applied loading force via the force constant k of the cantilever, the loading force can be written as

$$F = kd = k(z - \delta) \tag{1}$$

The elastic deformation of two spherical surfaces touching under load was calculated theoretically in 1882 by H. Hertz.[22] Sneddon[23] extended the calculation to other geometries, like a cone pushing onto a flat sample as used here. We will still call this model a Hertz model, to distinguish it from others, including other effects such as adhesion or plastic deformation.

The Hertz model gives the following relation between the indentation δ and the loading force F

$$F = (2/\pi)[E/(1 - \nu^2)]\delta^2 \tan(\alpha) \tag{2}$$

Here, E is the elastic or Young's modulus, ν is the Poisson ratio of the sample, and α is the half opening angle of the indenting cone.

To calculate the Young's modulus, we apply a fit of the Hertz model to the curve that is calculated as the average value of the approach and the retract trace of each force curve. Combining eqs 1 and 2 yields

$$z - z_0 = d - d_0 + \sqrt{\frac{k(d - d_0)}{(2/\pi)[E(1 - \nu^2)] \tan(\alpha)}} \tag{3}$$

The zero deflection d_0 has to be determined in the noncontact part of the force curve. Because the gelatin network obeys rubber elasticity, we assumed a Poisson ratio of 0.5. We used 8 mN/m as the determined force constant and 18° as the half-opening angle of the cone, corresponding to the specifications of the manufacturer. So, two quantities in eq 3 are unknown: the contact point z_0 and the Young's modulus E. These quantities can be determined independently by taking two different deflection values and their corresponding z values from a force curve, as shown in Figure 1. These two data points then define the range of deflection values, corresponding to the range of loading force, in which the Hertz model is applied. We will call that range in our later discussion range of analysis.

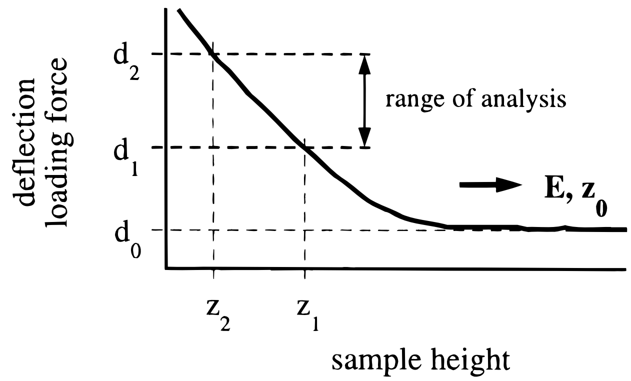

Figure 1. Force curve on a gelatin film. The average data of approach and retract trace are plotted. Parameters necessary for data analysis are depicted in the graph: the zero deflection (force) d_0 and the range of analysis defined by two data points (d_1, z_1) and (d_2, z_2). The analysis as described in the text yields independently the contact point z_0 and the Young's modulus E.

Because the AFM tips used are rather sharp cones, the induced shear stress is on the order of the sample's Young's modulus[16] with the danger of producing plastic deformation. To prove the elastic behavior of the gelatin, we also recorded hundreds of force curves on the same location of the sample and observed neither a change of the elastic properties nor a hysteresis within a single force curve.

Force Mapping. Typical parameter settings used for data acquisition in the force mapping mode were that each force map consisted of 32 × 32 force curves over a lateral scan size of 15 μm. Data were recorded in relative trigger mode at a rate of 7.1 force curves per second. The force maps were analyzed using the program IGOR (Wavemetrics, Lake Oswego, OR) on a Macintosh computer.

Results

To obtain the elastic properties of the investigated area of a sample, the force-mapping mode was employed: force curves were recorded while the tip was raster scanned laterally over the sample. The result is a so-called force map. Several quantities can be calculated from these force curves, such as adhesion and topography. Figure 2 shows force mapping data of a gelatin wedge as used in this study. Figure 2a shows the topography as calculated from the point of contact in each force curve. Each little square in the map represents the location of one force curve. Figure 2b shows the topographic profile along one line of Figure 2a. This cross-section of the topography of the sample shows that the wedge has a very homogeneous slope. Additionally, the edge of the film is well pronounced, which was important for recording the reference curves on the substrate that are necessary for calibrating the deflection signal within each force map. The arrows mark the position of three force curves that are shown in Figure 3. The curves are taken at locations of different film thicknesses of 150 and 410 nm and 1.15 μm. Depending on the indentation of the tip into the sample, the cantilever senses the underlying stiff substrate at small film thicknesses. Thus, the force curves become steeper with decreasing film thickness.

To test the accordance of the Hertz model we compared the indentation in the experimental data with the prediction of the theory. Figure 4 shows two characteristic force curves that were taken at two locations of the same soft gelatin wedge with a different film thickness. One was recorded at a film thickness of 1.4 μm (thick trace in Figure 4), the other at a thickness of 180 nm (thin trace in Figure

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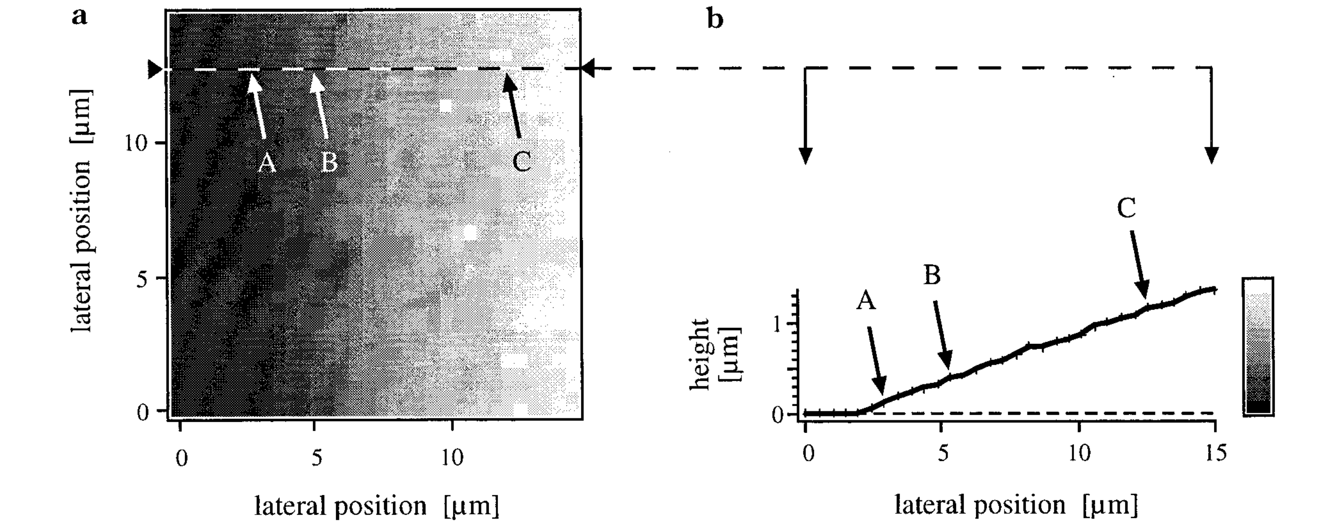

Figure 2. (a) Force map of a gelatin wedge on a glass substrate. Each square in the map represents the location of one force curve. (b) The topography of the sample along one line of the force map is obtained by analyzing the point of contact in each force curve. The position of three force curves, which will be shown in Figure 3, are marked with arrows.

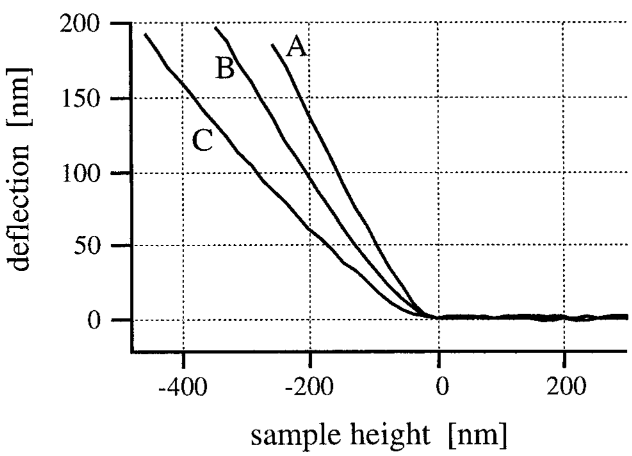

Figure 3. Three force curves taken at locations of a film thickness of 150 nm (curve A), 410 nm (curve B), and 1.15 μm (curve C). At high forces, the force curves are steeper for small thicknesses because the cantilever deflection is influenced by the underlying stiff substrate at these small film thicknesses. For comparing the slopes more easily, the curves are shifted such that their points of contact coincide.

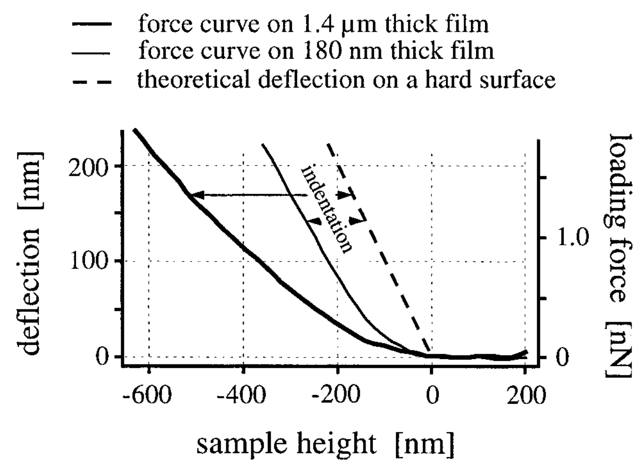

Figure 4. Two characteristic force curves that were taken on the same soft gelatin wedge but at two locations with a different film thickness of 1.4 μm (thick trace) and 180 nm (thin trace). To calculate the indentation, the measured deflection of the force curve has to be subtracted from a force curve on a stiff sample, where no or negliable sample indentation occurs (dashed line).

4). For comparison, we have added a theoretical force curve on the substrate (dashed line). The indentation of the force curves can be calculated as the difference between the experimental curve and a theoretical curve with slope one that corresponds to a force curve recorded on an infinitely stiff sample. The film thickness is given by the difference between the contact points of force curves on the substrate and on the gelatin film. However, here we have shifted the two curves in such a way that the contact points coincide.

To compare the experimental curves with the Hertz model, the indentation was calculated for both force curves. The result is plotted on a log−log scale in Figure 5. In the case of the thick film (Figure 5a) the prediction of the Hertz model with a Young's modulus of 44 kPa matches the experimental data over a wide range. The deviation for indentations <80 nm can be explained with the more spherical geometry at the end of the AFM tip, as already discussed in previous work,[10] because the typical radius of curvature is 20 nm according to the manufacturer's specifications. The slight deviation at indentations >300 nm can be explained by the shape of the cantilever tips; that is, they have a larger opening angle from 0.2 μm upward, which makes it more difficult for the tip to indent further.

In the case of the thin film (Figure 5b), the behavior is very different: the recorded indentation is smaller than the theoretical prediction of Figure 5a. The cantilever tip already "feels" the underlying stiff substrate. Even when using larger values for the Young's modulus (dashed line), the fit does not match the data well. The indentation behavior on this very thin and very soft film on the hard substrate is not described adequately by the Hertz model.

We can conclude that for sufficiently thick films, the Hertz model describes the experimental data well. In our case, this holds true even for thicknesses of the gelatin wedge of only 1.4 μm, although the maximum indentation is nearly 400 nm.

We will now discuss the effects of small thicknesses in more detail. Figure 6 shows a force curve recorded on a gelatin film with a thickness of 1.1 μm. The data are fitted by the Hertz model in various ranges of cantilever deflection (i.e., in various ranges of loading forces). For clarity we have plotted the experimental data three times

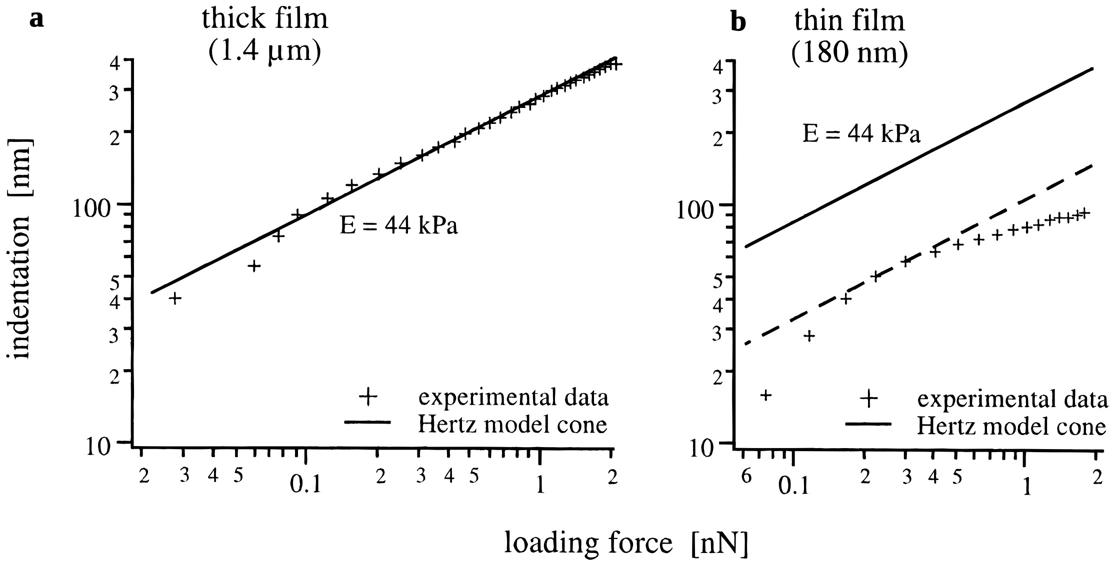

Figure 5. log−log plot of the indentation versus the loading force of the two force curves shown in Figure 4. In the case of the thick film (5a), the prediction of the Hertz model works well for large indentation values. Indenting the thin film (5b), the predicted indentation is bigger than the measured values. Even with a higher Young's modulus (dashed line), it is impossible to fit the whole range of data.

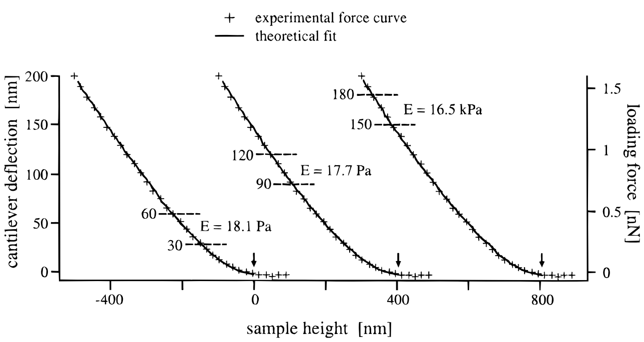

Figure 6. The Hertz model was applied in three different ranges of analysis to one experimental force curve that was taken at 1.1 μm thickness of the gelatin film. The theoretical force curves are superimposed on top of the experimental curve. For better visualization, the experimental curve is plotted three times shifted against each other. Independent from the range of analysis, the theoretic curves fit the experimental force curve very well. The calculated contact points, which are marked by the arrows, and the Young's moduli are nearly independent of the range of analysis.

and superimposed the theoretical curves on top of the data. Nevertheless, the calculated Young's modulus changes only little. The theoretical curves match the data nicely even outside the range of analysis. In addition, the calculated contact points vary only marginally. There is only a small fluctuation in the calculated Young's modulus, which has been summarized in Table 1 for various ranges of analysis. It is remarkable that even the fit in the lowest range, between 10 and 30 nm cantilever deflection, produces a good result of the Young's modulus. These values correspond to loading forces of 80 and 240 pN, respectively, and represent the very beginning of the useful fit range in Figure 5.

If the film thickness is small compared with the indentation, the situation is different. Figure 7 shows a force curve taken on the same gelatin wedge as the force curve in Figure 6 but at a film thickness of only 120 nm. This figure shows parts with different characteristics regarding the slopes. Immediately after the point of contact, the curve is flat corresponding to the elastic properties of the soft film. When the film gets compressed

Table 1. Different Ranges of the Applied Hertz Fit and the Respective Young's Modulus in the Case of a 1.1 μm Thick Soft Film[a]

range of analysis cantilever deflection, nm	mean applied loading force, nN	calculated Young's modulus, kPa
10−30	0.16	15.9
30−60	0.36	18.1
60−90	0.60	16.7
90−120	0.84	17.7
120−150	1.08	18.4
150−180	1.32	16.5
170−200	1.48	18.5
40−160	0.80	17.3

[a] The resulting values show no dependency to the applied range of analysis.

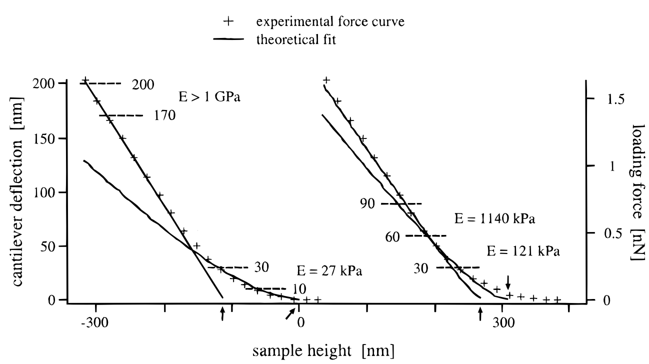

Figure 7. If the force curve is taken on a film that is thin compared with the indentation of the tip (in this case 120 nm), the calculated Young's moduli strongly depend on the chosen range of analysis. Only at lowest loading forces we can correctly determine the elastic properties of the thin film. Otherwise, the tip senses the underlying substrate and at very high loading forces the force curve approaches the slope of unity, corresponding to a force curve taken on the stiff substrate. Also the calculated contact point (small arrows), which is the measured height of the film, depends largely on the range of analysis.

more and more by applying an increasing loading force, the underlying stiff substrate is influencing the cantilever deflection. The slope of the force curve increases until it reaches a value of unity because further compression of the sample is not possible anymore. Consequently, there is a big influence of the chosen range of analysis on the

Table 2. Different Ranges of the Applied Hertz Fit and the Respective Young's Modulus in the Case of a Soft Film with Only 120-nm Thick Soft Film[a]

range of analysis cantilever deflection, nm	mean applied loading force, nN	calculated Young's modulus, kPa	distance of contact points, nm
10−30	0.16	27	0
20−50	0.28	54	19
30−60	0.36	121	48
40−70	0.44	331	69
60−90	0.60	1140	88
90−120	0.84	3280	99
120−150	1.08	8760	105
160−190	1.40	38200	111
170−200	1.48	>1 GPa	124

[a] The apparent Young's modulus increases over several orders of magnitude when increasing the range of analysis. At small deflections, corresponding to small loading forces, the elasticity of the thin film is probed. When increasing the loading forces the influence of the underlying stiff substrate becomes apparent.

calculated Young's modulus and on the contact point as calculated by the Hertzian fit. The results are summarized in Table 2: the apparent Young's modulus rises rapidly when increasing the loading force, mainly representing either the properties of the thin film (small deflection and small loading force) or the properties of the system thin film on stiff substrate (large deflection and large loading force).

The rising apparent stiffness of the sample corresponds to the increasing influence of the stiff substrate on the measurement. Consequently, it is necessary to choose a very low range of analysis to characterize the elastic properties of a thin film or to calculate a sensible contact point. The Young's modulus of the thin film approaches the value of the thick film for the lowest force range of analysis and finally reaches the same value within an error of ∼50% (Figure 8).

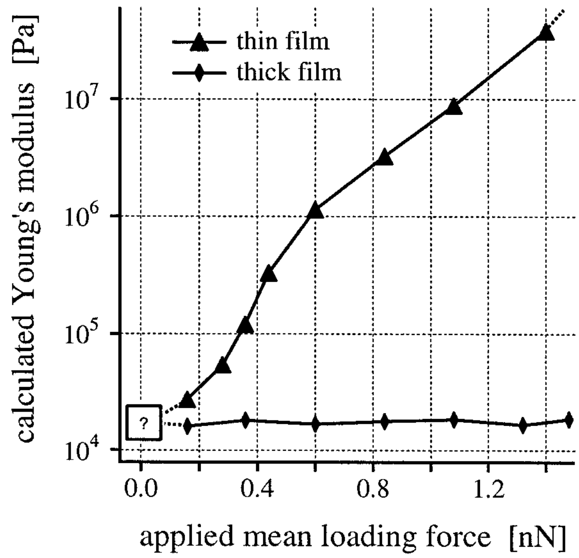

Figure 8. The calculated Young's modulus of the force curves of Figures 6 and 7 is plotted on a logarithmic scale versus the applied range of analysis (i.e., the mean value of the used range of loading forces). For the thick film, we see virtually no dependence of the calculated Young's modulus on the range of analysis; which is a consequence of the good match of the Hertz model to the experimental data as already discussed. In the case of the thin film, the large apparent Young's modulus of the sample for high loading forces is a consequence of the rising influence of the stiff substrate to the measurement. Only for very small forces does the calculated Young's modulus approach the value of the thick film within an accuracy of 50%. The calculated Young's modulus might even reach the value of the thick film if the applied loading forces would approach zero. Therefore, we can conclude that our analysis gives a reasonable estimate of the Young's modulus.

Discussion

The investigation of gelatin wedges as a model system show all the characteristics of thin and soft samples we have already mentioned. We have calculated the Young's modulus for different ranges of analysis that correspond to different deflection values and loading forces. In Figure 9 we have plotted the average of the calculated Young's moduli of three adjacent lines of the force map on a logarithmic scale as a function of the lateral position. Additionally, the corresponding height profile of the film is shown in the lower trace.

At thicknesses >300 nm, the wedge seems to have homogeneous elastic properties: independent from the chosen range of analysis, thus from the indentation of the tip into the sample, the calculated Young's moduli are similar and drop to <20 kPa. This result matches nicely the values reported in the literature for comparable macroscopic gelatin samples.[24,25] It is remarkable that in this part of the wedge that is already thicker than the indentation of the tip, the calculated Young's modulus is still decreasing, although its value is independent of the range of analysis. This continued decrease is surprising because in this range, the indentation behavior into the sample can be modeled very precisely with the Hertz model. However, there is still a decrease in the apparent Young's modulus at a thickness of 1 μm that will be discussed later.

With decreasing film thickness, the branches split. Depending on the applied loading force, the tip begins to "feel" the inhomogeneous elastic properties of the sample. Thus, at high values of the range of analysis (i.e., loading forces), the soft gelatin film is compressed entirely by the tip. This total compression is the reason for the high apparent Young's modulus of the thin film up to several megapascal.

In the application of the discussed method of analysis we can distinguish two different regimes concerning the calculated quantity of the Young's modulus. If the film is thick compared with the indentation of the tip, the Hertz model describes the force curves well and consequently the calculated contact point and Young's modulus are independent of the chosen range of analysis. Thus, the analysis produces "good" values. In our case, this regime is valid for the discussed force curves on the thick film of Figures 5a and 6. In the case of a thin film, the sample gets compressed in a way that the indentation behavior of the tip is already influenced by the underlying substrate. The inhomogeneous elastic properties result in the changing slope characteristics of the force curves as discussed in the context of Figure 7. Because the Hertz model fails at those small thicknesses, the chosen range of analysis plays an important role in analyzing the elastic properties of the sample. But, as has been shown, the model is capable of giving an estimate of the Young's modulus when applying very low loading forces. In any case, the calculated values will always represent an upper limit of the exact Young's modulus of the sample.

The monotonic decrease of the calculated Young's modulus in the thick range of the gelatin wedge needs further discussion. A possible explanation could refer to

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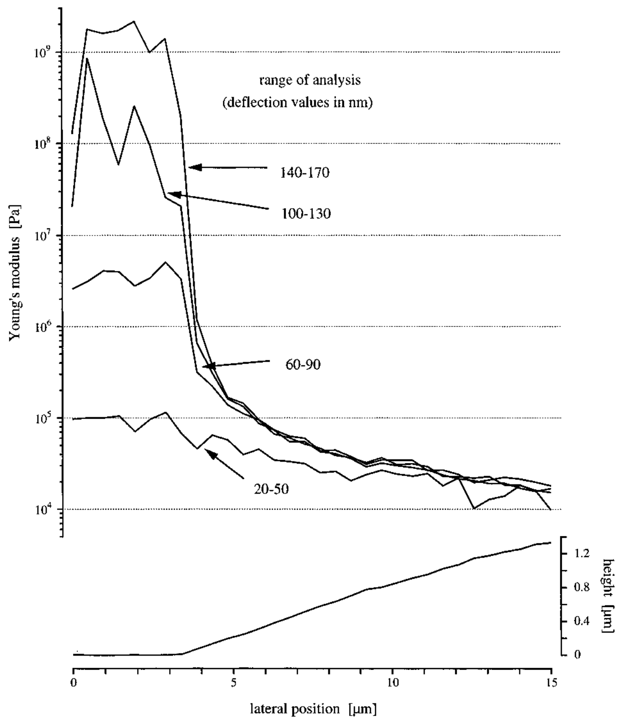

Figure 9. Calculated Young's modulus as a function of film thickness. For each force curve we calculated the Young's modulus within four different ranges of analysis and averaged the results of three adjacent lines. While converging in the bulk regime of the wedge, the branches of the different ranges of analysis split with decreasing thickness of the wedge. Because there is a thin film of gelatin also on the cantilever tip, there is a big dependence of the apparent Young's modulus on the applied range of analysis in the area of the bare substrate.

the entropic nature of rubber elasticity; that is, the individual polymer chains in rubber behave as Gaussian chains. There is no internal spring in the polymer, only the number of possible conformations determines its elastic properties. When reducing the number of conformations of a single strand (e.g., by pulling on it), an entropic price has to be paid. This decrease in entropy is the reason for the stiffness or elasticity of the polymer strand.[26] In our case, by building a wedge of polymer, we confine the polymer molecules between two surfaces: one being the substrate, the other the liquid on top. This confinement is known to cause significant changes in the organization of the affected polymers, with the consequence that the number of conformations of these molecules is reduced and the number of possible cross-links is increased.[27,28] Because the Young's modulus rises with an increasing density of cross-links,[26] the confined polymer has a larger Young's modulus than the bulk material at the same nominal density. This relationship may explain the observed decrease in Young's modulus at intermediate thicknesses.

However, another effect needs to be explained; that is, the Young's modulus of glass is 70 Gpa, whereas in the data shown the measured modulus of the glass slide without gelatin film varies depending on the applied range of analysis. This effect appears already after the first force scans in swollen gelatin and is stable then. We conclude that this effect corresponds to a thin film of gelatin that adheres to the tip. However, this film behaves like the gelatin sample on the substrate and thus does not falsify the analysis as presented here.

The behavior of the analysis around the edge of the film leads to important conclusions. On one hand it is necessary to apply a high loading force onto the substrate to completely compress the film that might adhere to the tip. This method is the only chance to get a correct value of the height and a slope of unity on the stiff substrate, which is necessary for calibrating the whole force map. On the other hand, analysis with very low ranges of loading force gives the opportunity to calculate the height and the elastic properties of a film even with a thickness of only a few tenths of nanometers.

Conclusions

We have investigated the elastic response of thin soft films of gelatin. If the film is >1 μm and the Young's modulus is >20 kPa, the Hertz model matches the data over a wide range of loading forces. However, for thinner films, there is a disagreement between the model and the experimental data. With these ultrathin films, the hard underlying substrate is sensed through the film, even at the lowest loading forces. However, the calculated Young's modulus is only off by 50% and can thus serve as an upper estimate for the real Young's modulus. If the force sensitivity of AFM could be increased (e.g., by using softer cantilevers), then the range of thicknesses and softnesses to which a Hertzian fit can be applied could be extended. In future work we will try to develop new models beyond the Hertzian model to describe the elastic behavior of ultrathin films more accurately. Possible routes could employ finite element methods.

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